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Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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Remembering Rollin Hotchkiss (1911–2004)

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ROLLIN HOTCHKISS, the distinguished biochemist and geneticist, died last December at the age of 93. I met Rollin in the summer of 1945 in Cold Spring Harbor. He had come to take the phage course, which was being offered for the first time that year. He returned as a summer investigator for many years thereafter, and during those summers I came to know him well and to admire him as a superb scientist and an exceptionally fine human being. The last time I saw Rollin was at a gathering to celebrate his 85th birthday, a festivity arranged by his wife, Magda Gabor-Hotchkiss, in Lenox, Massachusetts, where they had lived since 1982. The outpouring of affection and appreciation expressed there by his family, friends, colleagues, and former students was overwhelming. In the same measure that he was loved, he will be missed.

Rollin had a brilliant, analytical mind and a lively scientific imagination. He was kind, generous, and empathic, with a ready wit and an irrepressible sense of fun. He could summarize a symposium masterfully and then go on to lampoon its more contentious moments in hilarious original verse. At a conference, he could offer a penetrating critique of a young scientist's vulnerable conclusions so gently that it would come across as constructive advice rather than as a devastating embarrassment. He had sterling personal and scientific integrity and a genuine concern for the social consequences of science. He was considerate and respectful of every person he encountered.

I remember Rollin most vividly as he was during those summers at Cold Spring Harbor. Many of the movers and shakers of the exploding field of molecular genetics spent part or all of their summers there, and Rollin was always in the thick of the exciting nonstop talk that took place in the labs, on the beach, and at meals. His own work was at the leading edge and was much discussed, but he always seemed more intensely interested in knowing what others were doing and thinking, be they scientific stars or lowly graduate students. It was not all serious, however, and humor ran high. Rollin's playfulness was legendary, and I remember the inspired nonsense he created at the mock "graduation ceremonies" that took place every summer at the end of the phage course (Susman 1995). He brought the house down with his performance as the decrepit "oldest living alumnus" of the course, a role that he had earned by having taken the course the first time it was offered.

Early in his research career, Rollin established beyond doubt that DNA, and not protein, is the genetic material in bacteria. He was an active player in the molecular biology revolution, and his research contributed to a broad range of fields. Although he was best known for deconstructing the process of genetic transformation in bacteria, he also worked on chemical immunology, cytochemistry, antibiotics structure and mode of action, surface-active antiseptics, enzymes in subcellular particles, and chemistry of nucleic acids; in collaboration with others, including his wife, he worked on mutant exonucleases, genetic recombination, bacterial protoplast fusion, and control of chromosome expression. He was interested in the history of genetics and wrote about it extensively.

Born in South Britain, Connecticut, Rollin developed an early interest in science that was encouraged in high school. After his score topped all 14,000 high school students taking a nation-wide academic achievement test, his principal drove him to New Haven and saw to it that he was enrolled at Yale University. He was graduated from Yale in 1932 with a B.S. in chemistry, and only 3 years later he received a Yale Ph.D. in organic chemistry. He then joined the Rockefeller Institute (now Rockefeller University) in New York City, which remained his base throughout his career. Starting as a fellow, he advanced steadily in rank, becoming member and professor in 1955 and then professor emeritus at his retirement in 1982, when he and his wife moved to Lenox.

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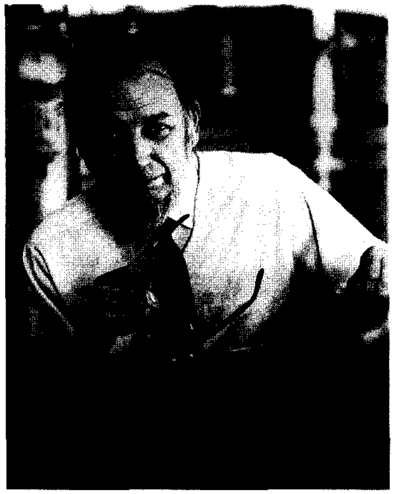
Rollin D. Hotchkiss at a conference at Cold Spring Harbor, 1949. Photograph courtesy of the Cold Spring Harbor Laboratory Archives.



Rollin D. Hotchkiss at Rockefeller University, 1975. Photograph courtesy of the Rockefeller University.

During the years from 1958 to 1990, he held visiting professorships at numerous scientific centers, both in the United States and abroad (England, France, Hungary, and Australia). In 1983 he was made adjunct professor of biology at the State University of New York at Albany, holding that position until 2002.

For his outstanding and wide-ranging contributions to science, Rollin received many honors, including election to the American Academy of Arts and Sciences, the National Academy of Sciences, the Hungarian Academy of Sciences, and the Royal Danish Academy. He was the recipient of honorary doctor of science degrees from Yale University, the University of Paris, the State University of New York at Albany, and Rockefeller University. He was also honored as the recipient of many awards and medals. He was president of the Harvey Society in 1958 and 1959 and of the Genetics Society of America in 1971 and 1972.

Rollin served on numerous scientific national advisory committees, including several under the National Institutes of Health, the National Science Foundation, and the National Academy of Sciences, in addition to those of the Biology Department of Oak Ridge National Laboratory, the Roswell Park Memorial Institute, the American Cancer Institute, the Foundation for Microbiology, the Trustees of Cold Spring Harbor Laboratory, and the Waksman Foundation for Microbiology.

Rollin's appointment as a chemist at the Rockefeller Institute, where he was hired to assist Oswald Avery and Walter Goebel, began on July 1, 1935. Goebel, his immediate research chief, arranged for him to spend the summer at the Marine Biological Laboratory at Woods Hole and to take the physiology course there as a way to become better acquainted with biology. It turned out to be a memorable experience, which Rollin described in an unfinished autobiography as "almost too exciting to bear." He was an organic chemist, and this was his first exposure to a community of biologists, one that included many of the leading lights in the field. The lectures and courses were "a running commentary on the latest ideas and experiments in all fields of biology," he wrote. He was fascinated by the "wild variety and beauty of living creatures" and thrilled to learn that the biochemical processes going on in them are basically similar. He began to read widely and systematically in biochemistry and genetics.

One of the course lecturers was Heinz Holter, a summer investigator from Copenhagen, who described to the class his use of the clever techniques that he and Kai Linderstrom-Lang had developed for very sensitive analyses. Rollin visited Holter's laboratory and volunteered to be his lab assistant. This association led to his spending the 1937–1938 academic year as a Rockefeller Foundation Fellow at the Carlsberg Laboratory in Copenhagen, with Holter and Linderstrom-Lang, where (he wrote) his "personal and scientific life was incomparably broadened and deepened."

After his summer at Woods Hole, Rollin joined Avery and Goebel in their work on the immunochemistry of the polysaccharide capsules of pneumonia bacteria. During the next 2 years he made important contributions to this project and became familiar with the microbiology of pneumococci. Then followed his year in Copenhagen, where he worked on peptide bonds in globular proteins and learned avant-garde analytic techniques. Returning to Rockefeller in 1938, he spent several fruitful years of work with René Dubos on the isolation, characterization, and mode of action of antibiotics (Hotchkiss 1941) and with Albert Claude on the localization of enzymes in intracellular particles (Hogeboom *et al.* 1946).

By 1943, Rollin was becoming increasingly intrigued by the work going on in Avery's laboratory on pneumococcal capsule-type transformation. He knew by then that Avery and his colleagues Colin MacLeod and Maclyn McCarty had shown the active "transforming principle" to be deoxyribonucleic acid, or DNA. These investigators had been surprised by their own result and had gone to great pains to purify the active agent, and especially to eliminate contamination with protein. When the work was published soon afterward (Avery *et al.* 1944), it was received by most geneticists with interest, but with considerable skepticism (Lederberg 1994). Although Avery and co-workers had stated their conclusions more cautiously, they mentioned comments by others who considered the transforming principle comparable to genes and viruses. Indeed, the comparison was so apt that it challenged the geneticists' nearly universal conviction that the genetic material must be protein, a prejudice quite broadly and tenaciously held for almost another decade. The contemporaneous description of DNA structure as a simple repeating tetranucleotide made it especially difficult to believe that it could be the genetic material.

In 1946, Rollin rejoined Avery's laboratory to participate in the research on pneumococcal transformation with Avery and his postdoctoral associate Harriett Taylor (later Ephrussi-Taylor). The three worked closely together until Taylor left the following year. After Avery retired in 1948, it was up to Rollin to carry on the work on transformation at Rockefeller. Almost immediately after starting his work on transformation, Rollin showed that purified or crystalline serum albumin could replace chest fluid from infected patients as an essential ingredient of the medium in test tube transformation experiments (Hotchkiss and Ephrussi-Taylor 1951). He then confronted the lingering doubts about the purity of the transforming DNA. Although Avery *et al.* (1944) had not been able to detect protein in their purified DNA preparations, there was persisting skepticism on the part of some biochemists and geneticists who believed that miniscule amounts of protein could be responsible for transforming activity. Rollin set out to answer this question once and for all. The amino acid nitrogen recoverable from hydrolyzed transforming DNA, prepared as in Avery *et al.* (1944), was indicative of no $>0.5\%$ protein as a contaminant. By 1949 Rollin was able to show that 100% of this amino acid nitrogen came from glycine produced in the decomposition of adenine and that a conservative estimate placed possible protein contamination at 0.02% at most; no protein at all was detectable by an extremely sensitive method. He presented this important result at several conferences, but did not publish it until a few years later (Hotchkiss 1952).

Rollin made some interesting observations in 1948 while analyzing the base composition of DNA with a sensitive new technique, paper chromatography (Hotchkiss 1948). He was able to obtain essentially quantitative recovery of purified bases with his chromatograms, independently of the similar work that he later learned was under way in Erwin Chargaff's laboratory. He found that his purified pneumococcal DNA and calf thymus DNA differed in base composition and demonstrated their different patterns at a conference in Paris (Hotchkiss 1949). A bonus was that his analysis of calf thymus DNA revealed a fast-migrating form of cytosine, which he correctly suggested was 5-methylcytosine, a finding of interest in view of our current appreciation of the importance of methylated bases in DNA.

A major advance was Rollin's demonstration that bacteria sensitive to penicillin could be transformed to resistance by highly purified DNA from a resistant strain and that this trait was transferred independently of capsule type (Hotchkiss 1951). A few years later he described double-marker transformation, showing that multiple heritable traits could be transferred via donor DNA to recipient bacteria either together or independently, much like linked or unlinked Mendelian genes in genetic recombination (Hotchkiss and Marmur 1954). In the next three decades, Rollin's work greatly expanded and clarified our understanding of the transformation process and of many aspects of the biology and chemistry of DNA. During these years he often collaborated with his colleagues Julius Marmur (Marmur and Hotchkiss 1955), Maurice Fox (Fox and Hotchkiss 1957, 1960), or Alexander Tomasz (Tomasz and Hotchkiss 1964) and with his students Joan Kent (Kent and Hotchkiss 1964), Sanford Lacks (Lacks and Hotchkiss 1960), and Elena Ottolenghi (Ottolenghi and Hotchkiss 1960, 1962). His collaboration with his wife Magda Gabor-Hotchkiss led to more than a dozen joint publications. Only a few selected examples from this productive and prolific period in Rollin's research career are mentioned here.

Rollin discovered that some loci are complex in that different segments of the marker region, indicative of multiple sites for breakage and exchange, can be recovered in transformation (Fox and Hotchkiss 1957). In an analysis of the complex sulfonamide resistance locus, he found that different levels of sulfonamide permitted selective assay for different configurations of the three linked loci. The results supported a general picture of nonreciprocal transfer of segments of the incoming DNA into a replicating genome (Hotchkiss and Evans 1958).

An important finding was that active transforming DNA is released into the medium from pneumococcal cultures during growth and disintegration (Ottolenghi and Hotchkiss 1960, 1962). This suggested a mechanism for transfer of genes in the absence of a sexual mechanism. Later, Gabor and Hotchkiss (1966) described a new way to terminate exposure of recipient bacteria to marker DNA, a technique that enabled them to time the entry of individual linked markers. They showed that in a linkage group, S-d-b, the intact DNA entered in that sequence, and the markers were incorporated in the same order. They also noted marked differences in the efficiency with which various markers were incorporated.

Working with protoplast fusion in *Bacillus subtilis*, HOTCHKISS and GABOR (1980) and GABOR and HOTCHKISS (1983) used pedigree analysis to study complementation and recombination in thousands of single colonies derived from primary products of fusion. Up to 10% of these colonies carried markers from both of the multiauxotrophic parent strains and were therefore "biparental," without exhibiting complementation. The absence of complementation showed that only one chromosome in the heterodiploids was expressed. These primary fusion products were capable of yielding parental segregants and a variety of mixed recombinant genomes. Although the apparent diploidy lasted for only a few generations, it showed that the fusion procedure had new possibilities for exploring bacterial chromosome expression and recombination patterns.

In the early 1960s, Rollin began to be concerned about the implications of genetics for human welfare, and especially about the possibility in the future of modifying human heredity, a subject that Joshua Lederberg had already been urging geneticists to consider. At a symposium held in 1963, Rollin made a prediction that proved quite prophetic (HOTCHKISS 1965a). In response to Salvador Luria's suggestion that viruses or episomes might be used to introduce specific DNA into human cells, Rollin said: "I should like to develop a related idea that new episomes or viruses might virtually be designed . . . constructed by attaching to host DNA *in vitro* the heterologous or modified genic material. Whether this were done by recombination in cells or by enzymes in test tubes, it could be done repetitively, and expanded fairly generally also to cover many traits" (p. 40). If one replaced the term "episome" with its later version "plasmid," this would amount to a preliminary description of the gene cloning procedure developed ~10 years later (COHEN *et al.* 1973). At the same 1963 symposium, Rollin's remark that "the pathway (to bioengineering) will be built from a combination of altruism, private profit and ignorance" was widely quoted by others sharing his concern. He continued to speak and write about the promise and the hazards of genetic intervention as his own attitude evolved (HOTCHKISS 1965b, 1970). It was concerns such as these that led to the famous 1973 Asilomar Conference on the hazards of genetic engineering.

Rollin's delightful playfulness came out often in the most serious contexts, and I cannot resist describing something he said at the 1963 symposium on the control of human heredity. In the discussion, Kimball Atwood proposed that it might be possible to put the enzyme cellulase into humans, so that people could consume paper. Rollin later recalled that at the time he had picked up on this idea, saying spontaneously that "he could picture researchers cleverly seasoning their edible works to improve collegial relations—we might soon have colleagues thanking the authors: 'I enjoyed so much your last reprint—please send me everything you publish from now on!'" He edited this remark out of the printed version of his comments, because he had found that people tended to recall his playful statements more readily than his serious ones. Years later, Rollin found among his papers a letter from Tracy Sonneborn written in 1964 thanking him for sending a copy of his symposium contribution, but expressing considerable disappointment that he had chosen not to include that exchange in his discussion!

After his retirement, Rollin maintained contact with many of his fellow scientists, often submitting their manuscripts to prestigious journals. He volunteered his services to many of the libraries and cultural organizations in the Lenox area. He also pursued his many interests and creative activities, including mineralogy, lapidary work, gem faceting, cabinet making, photography, poetry, stained glass, computer programming, and computer graphics.

Most of Rollin's extensive versifying over the years was humorous. He enjoyed poking fun at scientific stuffiness or provoking laughter while celebrating the birthdays of colleagues and friends. In 1994, he wrote an uncharacteristically personal poem that seems to me to make a fitting end to this tribute:

TESTAMENT

We arrive upon this teeming earth
With a tiny yelp or two
But little else attends our birth
Except—to get us through,
We lean on parents for a while,
And "docs" and friends to ease our woes
—or others whom we may beguile:
you scoff?—but that's the way it goes!
In youth, by turns: aloof, gregarious
We seek a sense of balance;
We find temptations multifarious,
Unless we find some talents!
With luck, some teacher—blessed soul
Helps avoid a costly bungle
And spots our real vocational role,
Before we're lost in the jungle.
My guides were great; the times so tough,
My work became my pleasure-cup—
When the Depression got more rough

We'd buckle down, and measure up!
In my time, schools could lead the way,
Opening up a slice of life—
But now, alas! Schools have to pay
The heavy cost of strife.
The tools I picked up on life's beach
Are subtler far than speech or sword:
Though now they've moved beyond my reach,
They've brought me much of life's reward.
A great one was—I found my mate,
To share rebellion—and compliance,
And continue to participate
In ventures in our chosen science.
And so, for threescore years or so,
I wrestled with an ancient quiz:
Struggling so we all could know
What makes life the way it is;
The Answer's a full encyclopedia
But to say it in a simpler way
Demanded by the modern media
It all boils down to DNA!

R. D. Hotchkiss (1994)

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